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Development of Bioassays and Approaches for the Risk Assessment of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and Related Compounds

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Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related halogenated aromatic hydrocarbons (HAHs) are industrial compounds or by-products that have been identified as contaminants in almost every component of the global ecosystem. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic HAH, and studies in rodents have shown that this compound is a carcinogen. Analysis of environmental samples for HAHs has shown that these extracts contain complex mixtures of isomers and congeners, and this greatly complicates risk assessment due to the paucity of data available for most of the individual compounds. Extensive research has demonstrated a common receptor-mediated mechanism of action for TCDD and related toxic HAHs, and this has led to the development of a mechanism-based risk assessment approach for HAHs. Toxic equivalency factors (TEFs; relative potency compared to TCDD) have been developed for selected HAH congeners, and the TEF values can be used to determine "toxic equivalents" (TEQs) for HAH mixtures. In addition, several bioassays that use receptor-mediated end points have been developed and can be used directly to determine the TEQs for HAH mixtures. The applications of the TEF/TEQ approach for the risk assessment of HAHs are considerable, particularly with the conversion of complex analytical data into TEQs. However, there appear to be several limitations to this approach, particularly with PCBs because their potential nonadditive (antagonistic), interactive effects with "2,3,7,8-TCDD-like" compounds may invalidate the use of the risk assessment procedure for some environmental matrices.

Introduction

Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) (Fig. 1) are halogenated aromatic compounds that have been identified as contaminants in almost every component of the global ecosystem including the air, aquatic and marine sediments, fish, wildlife and human adipose tissue, milk, and blood (*1–5*). PCBs were widely used as industrial compounds and have entered the enviromnent via numerous pathways including direct leakage from industrial facilities or machinery that used PCBs, leakage from waste sites containing PCB fluids, accidents, and spills. In contrast, PCDDs and PCDFs are industrial by-products that are formed during the production of chlorinated phenols and their derived products, PCBs and other chlorinated organic compounds. PCDDs and PCDFs have also been identified as by-products from the combustion of municipal and industrial waste, wood, coal, and numerous other combustion processes. In addition, PCDDs and PCDFs

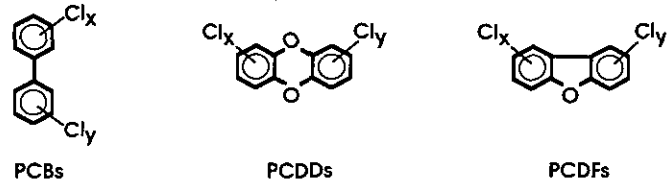

FIGURE 1. Structures of PCBs, PCDDs, and PCDFs.

are formed as by-products during the bleaching of wood and pulp paper, and residues of these compounds have been detected in pulp mill effluent and sludges, pulp samples, finished paper products, and aquatic sediments associated with discharges from pulp and paper mills (*1–7*).

The structures of individual PCB, PCDD, and PCDF congeners differ by their degree of chlorination and the ring substitution patterns, and Table 1 illustrates the large number of possible isomers and congeners for these chemical classes. There are 209 individual PCBs, and recent high-resolution analytical studies (*8*) have identified 132 of

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Table 1. Multiplicity of PCB, PCDF, and PCDD isomers and congeners.

	No. of C_1 atoms										
	1	2	3	4	5	6	7	8	9	10	Total
PCBs	3	12	24	42	46	42	24	12	3	1	209
PCDFs	4	16	28	38	28	16	4	1	–	–	135
PCDDs	2	10	14	22	14	10	2	1	–	–	75

these compounds in the commercial mixtures. The composition of the PCDD and PCDF by-products are highly variable and dependent on their source. For example, the PCDDs and PCDFs that have been identified as by-products of combustion are complex mixtures of isomers and congeners. In contrast, the PCDDs and PCDFs found as contaminants in many chlorinated phenol preparations are less complex and dependent on the structures of the chlorinated phenol precursors (*7*). For example, the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the major contaminant identified in the herbicide 2,4,5-T or Agent Orange (prepared from 2,4,5-trichlorophenol), which was used as a defoliant in Vietnam. Nevertheless, due to the diverse sources of PCDDs and PCDFs, most extracts of environmental samples contain complex mixtures of PCDDs, PCDFs, and PCBs.

Environmental Impact of PCBs, PCDDs, and PCDFs

PCBs were first detected in the environment in the mid- to late-1960s as unidentified peaks that were observed during the gas chromatographic analysis of environmental extracts for DDT and related metabolites and breakdown products (*9,10*). Subsequent studies have identified PCBs as residues in almost all environmental samples. Early packed-column gas chromatographic analysis of the commercial PCBs and PCB residues from diverse environmental extracts demonstrated the complex composition of these mixtures (*8–11*); moreover, recent studies using high-resolution analytical methods have confirmed that commercial PCB mixtures and the PCB residues in environmental extracts are highly complex. Inspection of the chromatograms of PCB residues from environmental extracts and from the commercial mixtures clearly demonstrates that the congener composition of the extracts does not resemble that of any single commercial PCB or their reconstituted mixtures. PCBs associated with atmospheric samples tend to contain some of the more volatile lower-chlorinated congeners, whereas the PCB composition of extracts from water and sediment samples can be highly variable and depend, in part, on the relative rates of microbial breakdown processes associated with these environments (*15–17*).

Safe and co-workers (*18*) first reported that the dominant PCB congeners (> 5%) in a composite human milk sample from Michigan were 2,4,4′-trichlorobiphenyl, 2,4,4′,5-tetrachlorobiphenyl, 2,3′,4,4′,5-pentachlorobiphenyl, 2,2′,3,4,4′,5′-hexachlorobiphenyl, 2,2′,4,4′,5,5′-hexachlorobiphenyl, 2,2′,3,3′,4,4′,5-heptachlorobiphenyl, and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl. These compounds constituted 58.9% of the total PCBs in the human milk extract; however, at least 55 different PCBs were identified. Ruarte-Davidson and co-workers (*19*) reported that a similar set of PCB congeners were identified in human tissue samples from several different locations, and many of these same compounds are major PCB components in wildlife samples. The differences in composition between the PCBs in various environmental samples is no doubt due to differential congener solubility, chemical and photochemical degradability, volatility, uptake, disposition, and metabolism. Thus, although samples from the same environmental matrix taken from different locations may contain a similar set of congeners, their relative composition will be variable.

PCDDs and PCDFs have also been widely identified in extracts of environmental samples and the composition of these analytes depend on their origins (e.g., air, water, sediments, or biota) and inputs from nearby sources (*1,2,20,21*). Several studies have reported the PCDD and PCDF composition of atmospheric samples from both urban industrial and rural areas (*22–28*). The absolute concentrations of the sum of PCDDs and PCDFs depend on local or regional inputs; however, the congener distribution pattern for most atmospheric samples resembles a typical combustion pattern for these compounds. Rappe and co-workers (*22,24*) reported the PCDD and PCDF content in several atmospheric particulate samples and only slight variations were observed: for example, the Σ PCDDs > Σ PCDFs; octachlorodibenzo-*p*-dioxin (OCDD) and heptachlorodibenzo-*p*-dioxin (HpCDD) were the dominant PCDDs with lower levels of the hexa-, penta-, and tetrachlorodibenzo-*p*-dioxins (HCDD, PeCDD and TCDD). In contrast, the tetra- and pentachlorodibenzofurans (TCDF and PeCDF) are the dominant PCDFs with lower levels of the octa-, hepta-, and hexachlorodibenzofurans (OCDF, HpCDF, and HCDF). Similar patterns of PCDD/PCDF distribution have been detected in soil samples (*29*) and on the outer surfaces of pine needles, which can be used as a biomonitor of atmospheric PCDDs, PCDFs, PCBs, and related organic contaminants (*30–32*).

In contrast, only the 2,3,7,8-substituted PCDDs and PCDFs are routinely detected in fish, wildlife, and human samples (*33–42*). The levels of PCDDs and PCDFs in biotic samples can vary depending on local contamination sources. In general, the pattern of these compounds in extracts from human samples resemble, in part, the combustion and atmospheric pattern, with OCDD being the dominant congener (Table 2). Although the analysis of fish samples also shows that only the 2,3,7,8-substituted compounds are routinely detected, there is at least one major difference in the congener distribution, namely, the low levels of OCDD in fish extracts. OCDD is routinely detected as a major component of aquatic and marine

Table 2. Distribution of PCDDs and PCDFs in environmental extracts (ppt).

Congener	Fish samples from Lake Michigan, range (36)	U.S. EPA study, mean ± SD (40)[a]
2,3,7,8-tetraCDD	3.0–4.4	14.1 ± 10.9
1,2,3,7,8-pentaCDD	7.0–12	18.9 ± 12.1
1,2,3,6,7,8-hexaCDD	4.1–10	163.9 ± 106.7[b]
1,2,3,7,8,9-hexaCDD	0.5–1.3	18.0 ± 118
1,2,3,4,7,8-hexaCDD	0.5–1.2	—[b]
1,2,3,4,6,7,8-heptaCDD	0.7–1.1	273.4 ± 248.4
octaCDD	0.8–1.2	1268 ± 1008
2,3,7,8-tetraCDF	31–39	2.1 ± 2.48
1,2,3,7,8-pentaCDF	3.9–8.0	0.6 ± 0.87
2,3,4,7,8-pentaCDF	8.1–14	23.0 ± 15.0
1,2,3,4,7,8-hexaCDF	0.9–2.4	21.4 ± 14.1
1,2,3,6,7,8-hexaCDF	0.7–1.9	10.9 ± 8.2
1,2,3,7,8,9-hexaCDF	<0.08	0.33 ± 0.33
2,3,4,6,7,8-hexaCDF	0.3–4.0	3.3 ± 2.7
1,2,3,4,6,7,8-heptaCDF	0.5–1.7	36.5 ± 25.6
1,2,3,4,7,8,9-heptaCDF	Not available	1.4 ± 1.2
octaCDF	<6.7	3.1 ± 4.1

[a]Adipose tissue samples.
[b]Given as a combined value of 1,2,3,6,7,8-hexaCDD/1,2,3,4,7,8-hexaCDD.

sediments (*43–45*) and the low to nondetectable levels in fish indicate that OCDD is not readily absorbed and/or retained in fish tissue. In summary, it is evident that the PCBs, PCDD, and PCDFs in the environment and human tissues are present as complex mixtures of isomers and congeners, and risk assessment approaches for these compounds must take into account this feature of congener multiplicity.

Common Biochemical and Toxic Responses and Mechanism of Action

Biochemical and Toxic Responses

The biochemical and toxic responses elicited by commercial PCBs, PCDDs, and PCDF mixtures and individual congeners are somewhat variable and can depend on the structure of the individual compound and the degree of chlorination of the PCB mixture. However, it has been demonstrated that specific mixtures and individual PCB, PCDD, and PCDF congeners elicit a comparable spectrum of toxic and biochemical responses in laboratory animals, humans, and mammalian cells in culture (*6,46–57*). For example, the halogenated aromatic hydrocarbons cause hepatotoxicity and porphyria, various endocrine effects, tissue-specific hypo- and hyperplastic responses, carcinogenic and anticarcinogenic effects, tumor promotion activity, immunosuppressive effects, reproductive and developmental toxicity, chloracne and related dermal lesions, and body weight loss. The number of biochemical responses caused by halogenated aryl hydrocarbons is constantly growing. Initial studies reported that this class of chemicals induce both phase I and phase II drug-metabolizing enzymes including aryl hydrocarbon hydroxylase (AHH), epoxide hydrolase, glucuronyl transferase, and glutathione *S*-transferase activities. In addition, the induction of several other responses, including ornithine decarboxylase, DT diaphorase, epidermal transglutaminase, aldehyde dehydrogenase, and δ-aminolevulinic acid synthetase have been reported. Halogenated aromatics also decrease a number of biochemical activities including the estrogen, progesterone, epidermal growth factor, and glucocorticoid receptors, uroporphyrinogen decarboxylase activity, thyroid hormone, and vitamin A levels. The effects caused by halogenated aromatics are also sex, age, species, and strain specific. For example, chloracne and related dermal lesions are typical observed in rabbits (ears), the skin of hairless mice, humans, and monkeys but are not seen in other strains of mice, rats, guinea pigs, and hamsters. The reasons for the differential responsiveness of laboratory animals and mammalian cells to halogenated aromatic hydrocarbons are not well understood.

Mechanism of Action of Halogenated Aromatic Hydrocarbon-Induced Responses

Poland and colleagues (*58–60*) and Thomas et al. (*61*) first recognized the differential responsiveness of several different aromatic and halogenated aromatic hydrocarbon congeners on the induction of hepatic microsomal AHH activity in genetically inbred aryl hydrocarbon (Ah) responsive and nonresponsive mice (typified by the C57BL/6 and DBA strains, respectively) and their backcrosses. Their results showed an approximately 10-fold difference in the response of these strains to the induction of AHH activity by 2,3,7,8-TCDD. Based on their results, it was proposed that the induction of AHH activity by 2,3,7,8-TCDD may be a receptor-mediated process and that the decreased Ah-responsiveness of DBA/2 mice was due to a defect in the Ah receptor in this mouse strain. Several studies with genetically inbred mice and their backcrosses have shown that for PCDDs, PCBs, and PCDFs, several toxic responses including immunotoxicity, teratogenicity, bodyweight loss, hepatotoxicity, and porphyria segregate with the Ah locus. Poland and co-workers first identified the cytosolic Ah receptor in hepatic tissues of C67BL/6 mice (*62*), and subsequent studies have confirmed the presence of this receptor in numerous animals, organs, and mammalian cells in culture (*63*). The Ah receptor is a high-affinity, low-capacity binding protein that exhibits saturable binding with 2,3,7,8-TCDD and other aromatic and halogenated aromatic hydrocarbons. The results of numerous studies demonstrate that the presence of the receptor is required for Ah responsiveness; however, there are many other factors that are also involved in the regulation of PCB-, PCDD-, and PCDF-induced responses. Nevertheless, the overall mechanism illustrated in Figure 2 is generally accepted as a model that describes the mode of action of these compounds. The molecular biology of 2,3,7,8-TCDD-induced *CYP1A1* gene expression (i.e., the induction of P4501A1-induced enzyme activities such as AHH and ethoxyresorufin *O*-deethylase [EROD]) has also been extensively investigated (*54,55,64–71*), and the results indicate that the nuclear Ah receptor complex acts as a ligand transcriptional factor (LTF) that

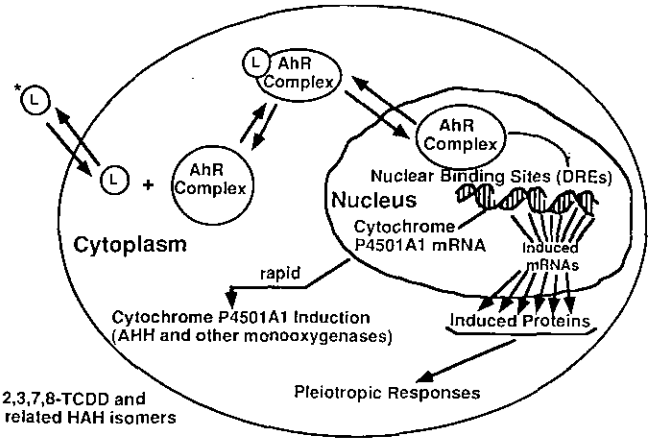

FIGURE 2. Proposed receptor-mediated mechanism of action for 2,3,7,8-TCDD and related compounds.

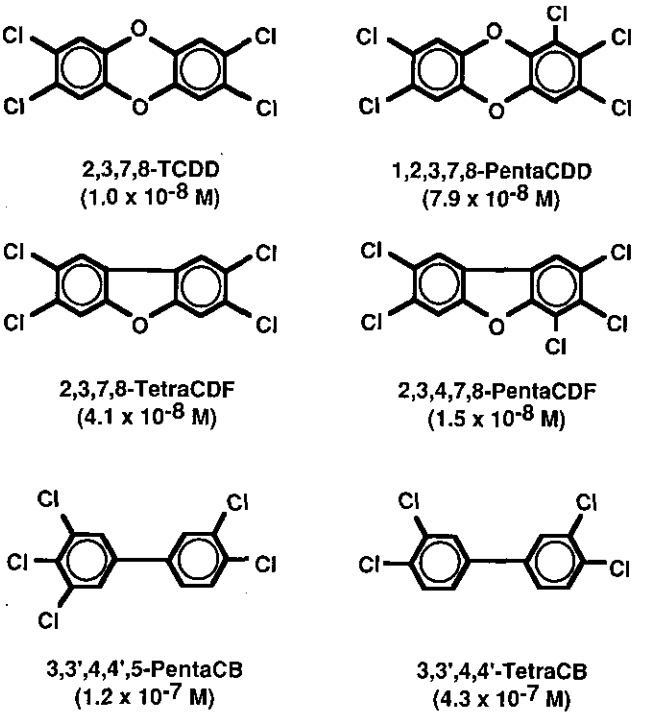

FIGURE 3. Structures and AH receptor binding affinities of selected toxic PCDD, PCDF, and PCB congeners.

binds to specific genomic sites in the 5′-region flanking the *CYP1A1* gene (dioxin responsive elements, DREs) and enhances gene transcription. This mechanism has been well documented for only a few responses such as the CYP1A1 and glutathione *S*-transferase *Ya* gene (*72*); however, the target genes involved in the toxic responses caused by this family of chemicals are unknown.

Structure–Function Relationships. One of the hallmarks of receptor-mediated responses is the unique structure–receptor-binding relationships observed for the interactions of structurally diverse ligands with the receptor. The structure-binding relationships for numerous PCB, PCDD, and PCDF congeners have been determined, and the results show that the most active congeners in competitive Ah receptor binding assays are those compounds substituted only in their lateral (2, 3, 7, and 8 for the PCDDs and PCDFs; 3, 3′, 4, 4′, 5 and 5′ for the PCBs) positions (*52,53,56,57*). Figure 3 illustrates the structures and competitive binding affinities of the most active congeners, which are all approximate isostereomers of 2,3,7,8-TCDD. The addition of nonlateral chlorine substituent or the removal of lateral chlorines tends to decrease the Ah receptor binding affinities for these compounds. For example, the mono-*ortho* coplanar PCB analogs exhibit 2,3,7,8-TCDD-like activity; however, due to the addition of the nonlateral *ortho* chlorine group, their potencies are substantially lower than the coplanar PCBs. It has also been reported that there was a rank-order correlation between the structure-binding and structure-activity relationships for diverse Ah receptor-mediated responses in several different species and in mammalian cells in culture. These observations support the proposed role of the Ah receptor in mediating the PCB, PCDD, and PCDF-induced responses caused by this family of environmental contaminants and form the mechanistic basis for the development of the toxic equivalency factor (TEF) approach for the hazard and risk assessment of halogenated aryl hydrocarbon mixtures.

Bradlaw and co-workers (*73,74*) first noted that the induction of AHH activity in rat hepatoma H-4II E cells was a potentially useful and sensitive *in vitro* bioassay for toxic halogenated aryl hydrocarbons. Additional studies by Safe and co-workers (*52,56*) have demonstrated that there is a good correlation between the structure-dependent AHH induction activities of individual PCB, PCDD, and PCDF congeners in rat hepatoma H-4II E cells and their *in vivo* toxic potencies in rats and mice. For example, Figures 4 and 5 illustrate the correlation between the *in vitro* AHH induction potencies of selected PCB, PCDD, and PCDF congeners in rat hepatoma H-4II E cells and their *in vivo* toxicities in the rat (*6,56*). These data confirm the utility of the *in vitro* induction assays as short-term test systems for the quantitative analysis of the toxicity of individual PCDD, PCB, and PCDF congeners and their mixtures. Moreover, these data and the results of numerous other studies also provide the essential mechanistic-based framework for the development of the TEF approach for the hazard and risk assessment of these compounds (*6*).

Development of TEFs for PCBs, PCDDs, and PCDFs

2,3,7,8-TCDD has generally been recognized as the most toxic halogenated aryl hydrocarbon, and the toxicity of several PCB, PCDD, and PCDF congeners relative to that of 2,3,7,8-TCDD has been reported (*6*). The results in Table 3 summarize the range of relative potencies of the

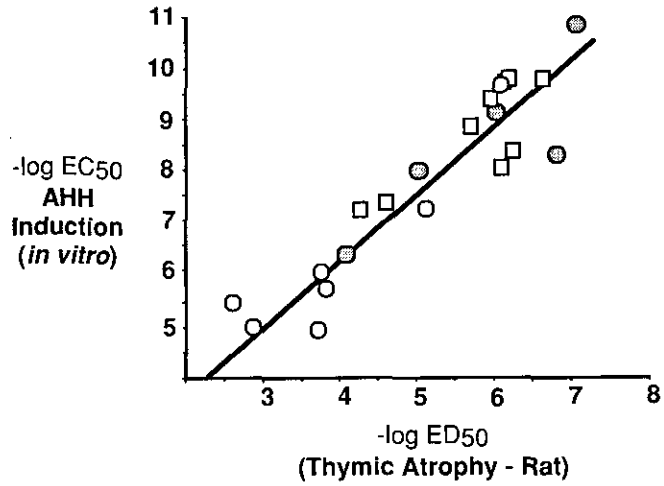

FIGURE 4. Correlation between the *in vitro* aryl hydrocarbon hydroxylase induction potencies versus the *in vivo* toxicity (thymic atrophy in the rat) for several PCDD (●), PCDF (□), and PCB (○) congeners (*6,56*).

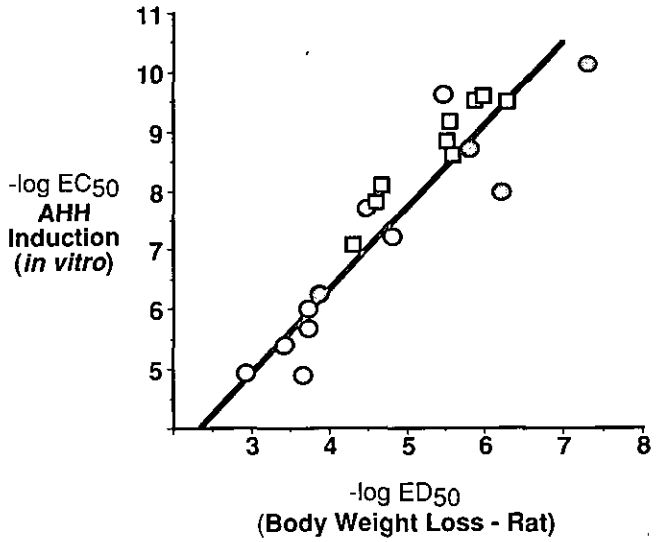

FIGURE 5. Correlation between the *in vitro* aryl hydrocarbon hydroxylase induction potencies versus the *in vivo* toxicity (body weight loss in the rat) for several PCDD (●), PCDF (□), and PCB (○) congeners (*6,56*).

more highly toxic PCDD, PCDF, and PCB congeners, which have been derived from numerous *in vivo* animal studies and *in vitro* bioassays (*6*). The results demonstrate that all of the compounds are less toxic than 2,3,7,8-TCDD and that for each congener there is a range of potencies relative to that of 2,3,7,8-TCDD. There is a wide variation in the range of these TEF values for different congeners, and the variability of the TEFs depend on several factors including the nature of the response, the animal or mammalian test system used, the route of exposure, and the duration of the experiment. The relative pharmacokinetics and metabolism of individual PCB, PCDD, and PCDF

Table 3. Relative range of toxicities of PCB, PCDD, and PCDF congeners compared to that of 2,3,7,8-TCDD (*6*).

Congener	*In vivo* responses	*In vitro* responses
2,3,7,8-tetraCDD	1.0	1.0
1,2,3,7,8-pentaCDD	0.59–0.053	0.64–0.07
1,2,3,6,7,8-hexaCDD	0.16–0.0152	0.5–0.005
1,2,3,7,8,9-hexaCDD	0.14–0.016	0.009
1,2,3,4,7,8-hexaCDD	0.24–0.013	0.13–0.015
1,2,3,4,6,7,8-heptaCDD	0.0076	0.003
octaCDD	>0.0013	0.0006
2,3,7,8-tetraCDF	0.17–0.016	0.43–0.006
1,2,3,7,8-pentaCDF	0.9–0.018	0.13–0.003
2,3,4,7,8-pentaCDF	0.8–0.12	0.67–0.11
1,2,3,4,7,8-hexaCDF	0.18–0.038	0.2–0.013
1,2,3,6,7,8-hexaCDF	–	0.48–0.037
1,2,3,7,8,9-hexaCDF	–	–
2,3,4,6,7,8-hexaCDF	0.097–0.017	0.1–0.015
1,2,3,4,6,7,8-heptaCDF	0.22	–
1,2,3,4,7,8,9-heptaCDF	0.20	–
octaCDF	–	–
3,3′,4,4′-pentaCB	0.3–0.0006[a]	
3,3′,4,4′-tetraCB	0.009–0.00008[a]	
3,3′,4,4′,5,5′-hexaCB	0.1–0.0012[a]	
Mono-*ortho* coplaner PCBs	0.00045–0.0000014[a]	

[a] *In vivo* and *in vitro*.

congeners may depend on the strain, sex, age, and species used and the target organ, and these factors no doubt contribute to the wide range of potencies of these congeners relative to that of 2,3,7,8-TCDD.

Nevertheless, several regulatory agencies have used these data to develop TEFs for the 2,3,7,8-substituted PCDDs and PCDFs (Table 4), which are routinely detected as by-products of industrial and combustion processes and as residues in fish, wildlife, and human samples (*75–79*). A conservative approach has been adopted for the selection of most of the individual TEF values, with particular emphasis on data obtained from long-term and reproductive studies. Safe (*6*) has also proposed a set of similar TEFs for these compounds in which the value for the 1,2,3,4,6,7,8- and 1,2,3,4,7,8,9-HpCDFs was increased to 0.1 due to the result of recent studies on the immunosuppressive effects of the compounds in C57BL/6 mice. Regulatory agencies have not yet developed TEFs for the 2,3,7,8-TCDD-like PCB congeners; however, tentative TEF values for the coplanar PCBs (Fig. 2) and their mono- and di-*ortho*-substituted analogs have also been proposed (*6*).

Applications and Limitations of the TEF Approach

One of the major applications of the TEF approach involves the conversion of analytical data into toxic or 2,3,7,8-TCDD equivalents (TEQs) in which Σ (congener concentration) × (congener TEF) = TEQs for that sample. For example, the results in Table 5 summarize the TEQs for the PCB, PCDD, and PCDF components of human milk fat extracts from Quebec, Canada (*80*). The total TEQs for the PCDDs, PCDFs, and PCBs were 9.34,

Table 4. Proposed toxic equivalents factors for the toxic halogenated aromatics (*6*).

Congener	TEFs proposed by Safe (*6*)	International/ EPA (*77,78*)	Nordic (*76,79*)
PCDDs			
2,3,7,8-tetraCDD	1.0	1.0	1.0
1,2,3,7,8-pentaCDD	0.5	0.5	0.5
1,2,3,6,7,8-hexaCDD	0.1	0.1	0.1
1,2,3,7,8,9-hexaCDD	0.1	0.1	0.1
1,2,3,4,7,8-hexaCDD	0.1	0.1	0.1
1,2,3,4,6,7,8-heptaCDD	0.01	0.01	0.01
octaCDD	0.001	0.001	0.001
PCDFs			
2,3,7,8-tetraCDF	0.1	0.1	0.1
2,3,4,7,8-pentaCDF	0.5	0.5	0.5
1,2,3,7,8-pentaCDF	0.1	0.05	0.01
1,2,3,4,7,8-hexaCDF	0.1	0.1	0.1
2,3,4,6,7,8-hexaCDF	0.1	0.1	0.1
1,2,3,6,7,8-hexaCDF	0.1	0.1	0.1
1,2,3,7,8,9-hexaCDF	0.1	0.1	0.1
1,2,3,4,6,7,8-heptaCDF	0.1	0.01	0.01
1,2,3,4,7,8,9-heptaCDF	0.1	0.01	0.00
octaCDF	0.001		
PCBs			
3,3',4,4',5-pentaCB	0.1		
3,3',4,4',5,5'-hexaCB	0.05		
3,3',4,4'-tetraCB	0.01		
Mono-*ortho* coplanar PCBs	0.001		
Di-*ortho* coplanar PCBs	0.00002		

Table 5. 2,3,7,8-TCDD equivalents (TEQs) in human adipose tissue samples, Quebec, Canada (*80*).

Congener	Toxic equivalents factor	Mean concentration, ppt	TEQs, ppt
2,3,7,8-tetraCDD	1.0	2.3	2.3
1,2,3,7,8-pentaCDD	0.5	4.8	2.4
1,2,3,4,7,8-hexaCDD	0.1	34.6	3.46
1,2,3,6,7,8-hexaCDD	0.1		
1,2,3,7,8,9-hexaCDD	0.1	6.4	0.64
1,2,3,4,6,7,8-heptaCDD	0.01	40.5	0.41
octaCDD	0.001	131.7	0.13
Total			9.34
2,3,7,8-tetraCDF	0.1	6.1	0.61
1,2,3,7,8-pentaCDF	0.1	–	–
2,3,4,7,8-pentaCDF	0.5	5.2	2.6
1,2,3,4,7,8-hexaCDF	0.1	3.3	0.33
2,3,4,6,7,8-hexaCDF	0.1	1.1	0.11
1,2,3,6,7,8-hexaCDF	0.1	2.3	0.23
1,2,3,7,8,9-hexaCDF	0.1	–	–
1,2,3,4,6,7,8-heptaCDF	0.1	4.5	0.45
1,2,3,4,7,8,9-heptaCDF	0.1	–	–
octaCDF	0.001	–	–
Total			4.33
3,3',4,4',5-pentaCB	0.1	80.5	8.05
3,3',4,4',5,5'-hexaCB	0.5	32.7	1.63
3,3',4,4'-tetraCB	0.01	8.1	0.08
2,3,3',4,4'-pentaCB	0.001	4400	4.4
2,3',4,4',5-pentaCB	0.001	17400	17.4
2,3,3',4,4',5-hexaCB	0.001	6.2	6.2
Total			37.76

4.33, and 37.76 ppt, respectively, and the overall percent contribution of these subclasses to the total TEQs in this sample were 18.1, 8.4, and 73.5%, respectively. These results are consistent with data from several other studies that indicate that the coplanar and mono-*ortho* coplanar PCBs are major contributors to the total TEQs in fish, wildlife, and human samples (*81–86*).

There is also evidence that the TEF approach can be used to predict the toxicity of PCDD and PCDF mixtures. For example, Eadon and co-workers (*87*) tested the toxicity of an extract that contained a complex mixture of PCDD and PCDF congeners. The high-resolution analytical data for these mixtures was converted into TEQs using the TEF factors and the TEQs for the PCDDs and PCDFs in the soot extract were 0.82 and 14.55 ppm, respectively. In parallel experiments, the extract was administered to guinea pigs and compared to the toxicity of 2,3,7,8-TCDD for several responses including thymic atrophy, body weight loss, increased serum triglycerides, decreased serum alanine aminotransferase levels, the formation of hepatocellular cytoplasmic inclusion bodies, and acute lethality. The results showed that for the soot extracts, ED_{50} values derived for these toxic responses from the *in vivo* studies was 19, 21, 5, 18, 10, and 2 ppm, respectively, with an average value of 12.5 ppm. The calculated TEQ from the analytical data using the TEF approach was 15.37, and this value was remarkably similar to the TEQs determined from the *in vivo* studies in guinea pigs. These data and results from other studies confirm the utility of the TEF/TEQ approach for the hazard and risk assessment of PCDDs and PCDFs.

The use of TEFs for the hazard and risk assessment of PCBs is also being considered as a regulatory tool for the hazard and risk assessment of PCBs (*88*). Structure–function relationship studies for PCB isomers and congeners have demonstrated that the coplanar and mono-*ortho* coplanar PCBs elicit 2,3,7,8-TCDD-like responses. The development of TEF values (Table 4) for these compounds is appropriate and should be useful for the hazard and risk assessment of the total TEQs in extracts of samples from environmental matrices. The TEF values that have been proposed for PCBs (*6*) may be highly conservative and should be confirmed or revised based on ongoing research on the toxicity of PCB isomers and congeners. Davis and Safe (*89*) have determined the dose-dependent immunosuppressive effects of several commercial PCBs in C57BL/6 mice. The calculated TEQs (from analytical data) for Aroclors 1260, 1254, 1248, and 1242 significantly overestimated (> 15-fold) the immunosuppressive effects of these mixtures. Thus, the interactive effects of the 2,3,7,8-TCDD-like PCB congeners with other PCB components of the mixture were not additive and appeared to be antagonistic. These results are consistent with the reported activity of individual PCBs and PCB mixtures as 2,3,7,8-TCDD antagonists (*89–92*) and suggest that the TEF approach may significantly overestimate the TEQs for environmental extracts containing PCB, PCDD, and PCDF mixtures in which the concentrations of the PCBs were > 100-fold higher than the PCDDs and PCDFs. Further studies are required to resolve this important issue.

Another major unresolved problem associated with the TEF approach for PCBs is related to the potential biochemical and toxic effects elicited by compounds that do not act through the Ah receptor. For example, recent studies have shown that a series of *ortho*-substituted PCBs that do not exhibit 2,3,7,8-TCDD-like activity, are neurotoxic in both rodent and monkey cells in culture (*93,94*). These compounds, which typically contain two to three *ortho*, zero to two *para* and low *meta* chlorine substituents, decrease the dopamine content in brain tissues and in mammalian cells. The adverse environmental and human health impacts of these more highly *ortho*-substituted PCB congeners are unknown and require further investigation.

A number of different structural classes of PCBs also induce P450 activities that are not mediated through the Ah receptor (*95,96*). For example, several individual congeners, including 2,2′,4,4′-tetraCB and 2,2′,4,4′,5,5′-hexaCB have been characterized as pure phenobarbital (PB)-type inducers and structure-induction studies suggest that compounds substituted in at least two *para* and two *ortho* substituents induce this type of monooxygenase enzyme activity. The potential adverse health effects of this class of PCBs has not been determined; however, like PB, these compounds cause hepatomegaly and exhibit activity as potential tumor promoters in short-term bioassays for carcinogenesis. For example, 2,2′,4,4′,5,5′-hexaCB promotes diethylnitrosamine-induced ATPase-deficient lesions in rat liver (*97,98*). Because the previous risk assessment of PCBs has been derived, in part, from carcinogenicity studies of specific PCB mixtures (*99,100*), it is important not only to assess the contribution of all structural classes of PCBs as carcinogens and anticarcinogens but also to determine whether PCB-induced carcinogenicity is due to the activity of these compounds as either promoters or initiators or a combination of the two. Thus, although a limited TEF approach may be warranted in some situations for assessing the risk of Ah receptor-mediated responses, the application of this procedure for PCB-induced carcinogenic effects requires further validation and more research.

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